J. Helminthol. Soc. Wash.
63(1), 1996, pp. 42–46

Relationships Between *Glypthelmins pennsylvaniensis* (Trematoda: Digenea) Infections and Host Size

ROBERT B. TUCKER AND JAMES E. JOY[1]
Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755

ABSTRACT: A total of 238 male spring peepers, collected from 3 different western West Virginia marshes during the spring breeding seasons of 1992, 1993, and 1994, were examined for *Glypthelmins pennsylvaniensis* infections. Prevalence was 66.4% with a mean intensity of 6.1. Hosts were divided into 5 sample populations, based upon year and site of collection, to examine relationships between 1) host weight and numbers of *G. pennsylvaniensis* individuals and 2) numbers of this digenean species versus their mean length. As infected host weight increased, the number of digeneans declined in all 5 host sample populations, but this inverse relationship was not significantly different from zero (i.e., $b = 0$) for 4 of those populations. Mean length of *G. pennsylvaniensis* individuals decreased as their numbers increased in a given host. This inverse relationship was significantly different from zero (i.e., $b \neq 0$) for individuals in all 5 host sample populations. Mean weights of infected hosts were significantly ($P < 0.05$) lower than mean weights of uninfected hosts in 3 of the 5 sample populations.

KEY WORDS: *Glypthelmins, Pseudacris,* West Virginia, spring peeper.

In this paper we examine some relationships between the digenetic trematode, *Glypthelmins pennsylvaniensis* (Cheng, 1961), and its amphibian host, the northern spring peeper, *Pseudacris c. crucifer* (Weid-Neuweid). Because prevalence of *G. pennsylvaniensis* can exceed 50% in spring peeper populations, with a range of 1–20 (sometimes more) digeneans per host individual (Coggins and Sajdak, 1982; Muzzall and Peebles, 1991; Joy and Dowell, 1994), this parasite/host model offers the advantage of establishing databases of sufficient size for statistical analyses with a reasonable collection effort. There is the added opportunity to study effects of limited space on digenean parasites with this model because the small intestine of spring peepers is relatively small. Rankin (1937), observing trematodes in the genera *Brachycoelium, Plagitura,* and *Megalodiscus,* noted that, "Crowding of many individuals within a small area may account for small size, for when these flukes occur in small numbers, they are much larger." Willey (1941) and Fried and Nelson (1978), working with *Zygocotyle lunata,* and Nollen (1983), studying *Philophthalmus gralli,* also demonstrated stunting of adult digeneans in crowded conditions. Cheng (1961), Coggins and Sajdak (1982), Muzzall and Peebles (1991), and Joy and Dowell (1994) have provided prevalence and mean intensity data for *G. pennsylvaniensis* in spring peepers from a wide geographic range (Pennsylvania, Wisconsin, Michigan, and West Virginia, respectively), but there is still much to learn about relationships between this digenean species and its amphibian host. In this paper we emphasize 1) differences between weights of infected versus uninfected hosts, 2) the relationship between host size (as weight) and number of *G. pennsylvaniensis* individuals present, and 3) the relationship between numbers of digeneans in a given host and their mean length in that host.

Materials and Methods

A total of 238 male northern spring peepers, *Pseudacris c. crucifer* (Weid-Neuwied), were collected from 3 marsh areas in western West Virginia during the breeding seasons of 1992 through 1994. Two of the marshes, Beech Fork (BF) and Shoals (SM), are in Wayne County (USGS Topographic Map, Lavalette Quad). The third marsh, Green Bottom Wildlife Management Area (GB), is in northern Cabell County (USGS Topographic Map, Athalia Quad). Hosts from these 3 sites were segregated into 5 sample populations based on period of collection. Populations designated BF92, SM93, and SM94 were examined in different years, so their separation seemed appropriate. Host populations from Green Bottom were taken from the same site in 1994 and could have been grouped; however, we chose to consider the Green Bottom material as an early breeding season population (GBM94) and a late breeding season population (GBA94).

All hosts were captured between 2000 and 2200 hours, placed in 4-liter screw-cap jars (no more than 10 host individuals per jar) with moist paper towelling, returned to the laboratory, and placed in a refrigerator at 4°C. All hosts were necropsied within 12–24 hr after capture. Immediately prior to necropsy, each host individual was weighed to the nearest 0.1 g and measured for snout–vent length (SVL) with vernier calipers to

[1] Corresponding author.

Table 1. Combined probabilities for mean body weights of *Pseudacris c. crucifer* individuals infected ($\bar{x}_i$) with *Glypthelmins pennsylvaniensis* compared with mean weights of uninfected ($\bar{x}_u$) hosts in 5 different West Virginia host sample populations.

Host pop.	N_i*	$\bar{x}_i$ (±1 SD)	N_u*	$\bar{x}_u$ (±1 SD)	t	df	P	ln P*†
BF92	22	1.53 (0.184)	21	1.70 (0.227)	2.698	41	0.021	−3.863
SM93	25	1.70 (0.250)	25	1.85 (0.228)	2.212	48	0.033	−3.411
SM94	29	1.82 (0.257)	5	1.86 (0.291)	0.317	32	0.763	−0.270
GBM94	55	1.67 (0.249)	24	1.82 (0.318)	2.273	77	0.029	−3.540
GBA94	27	1.46 (0.199)	5	1.47 (0.237)	0.106	30	0.916	−0.088

* N_i = number of infected hosts; N_u = number of uninfected hosts. ln P = natural log of probability value.
† Reject H_o; $\bar{x}_i = \bar{x}_u$ because the observed value of $-2 \Sigma \ln P$ (i.e., 22.344) > $X^2_{.05[10]} = 18.307$.

the nearest millimeter. Weight values, rather than SVL's, are used to describe host size throughout this paper because the former provided a wider range of values and thus a better estimation of host size.

After weighing, the host's spinal cord was severed at the base of the head with a surgical blade, and the small intestine was removed and examined.

Glypthelmins pennsylvaniensis individuals recovered from the small intestines of infected hosts at BF92 and SM93 were killed and fixed with 10% buffered formalin while under slight coverslip pressure. Length measurements of these *G. pennsylvaniensis* individuals were made with the aid of an ocular micrometer, and their mean length (±1 SD) was calculated. Living trematodes recovered from infected hosts at SM94, GBM94, and GBA94 were segregated by host and placed directly into a series of 3.5-mm petri dishes containing 10 ml of tap water and a drop of stock mentholated alcohol solution (Abdel-Malek, 1951). Each appropriately labeled dish, containing this relaxing fluid and trematodes from a single host, was then refrigerated at 4°C for 1 hr (Fried, 1962). After 1 hr of refrigeration in the menthol solution, immobile trematodes were killed and fixed in 10% buffered formalin, then transferred to fresh formalin for storage. These trematodes were subsequently measured for total length with the aid of an ocular micrometer (without coverslip pressure) while in formalin mounts, and mean length (±1 SD) was calculated.

Three null hypotheses were established for testing: 1) that mean weight of infected hosts was equal to that of uninfected hosts (i.e., H_o: $\bar{x}_i = \bar{x}_u$), 2) that there was no relationship between number of *G. pennsylvaniensis* individuals and size (as weight) of infected host (i.e., $b \neq 0$ for trematode numbers as a function of host weight), and 3) that there was no relationship between mean trematode length in a given infected host and the total number of trematode individuals in that host (i.e., $b = 0$ for mean trematode length as a function of total trematodes). The test statistic for the first hypothesis was a t-test, with a probability value for each host sample population given in Table 1. The test statistic for the latter two hypotheses was an F-test, with probability values given in the appropriate tables. Statistical tests were performed on an IBM compatible computer using Systat software (Systat, Inc., 1992).

Voucher specimens of *G. pennsylvaniensis* are deposited in the U.S. National Parasite Collection (Beltsville, Maryland 20705) under USNPC 83429.

Results

A total of 238 male spring peepers, representing 5 West Virginia sample populations, were examined for *Glypthelmins pennsylvaniensis* infections. Mean infected host weight was significantly lower ($P < 0.05$) than uninfected host weight in 3 of the 5 host populations (Figs. 1–5; Table 1). During the course of this investigation, 974 *G. pennsylvaniensis* individuals were recovered from 158 West Virginia spring peepers (Table 2). With the exception of the SM93 host population, there was no significant difference between mean trematode intensity levels (Table 2).

Larger infected hosts, in every host sample population, carried fewer *G. pennsylvaniensis* individuals than did smaller hosts. Still, this negative correlation between infected host weight and numbers of trematodes present was not significant (i.e., $b = 0$) for infected hosts in 4 of the 5 sample populations (Figs. 1–5; Table 3). Mean lengths of *G. pennsylvaniensis* individuals decreased as their numbers increased in a given host. This negative correlation was significant (i.e., $b \neq 0$) for all 5 host sample populations (Figs. 6–10; Table 4).

Discussion

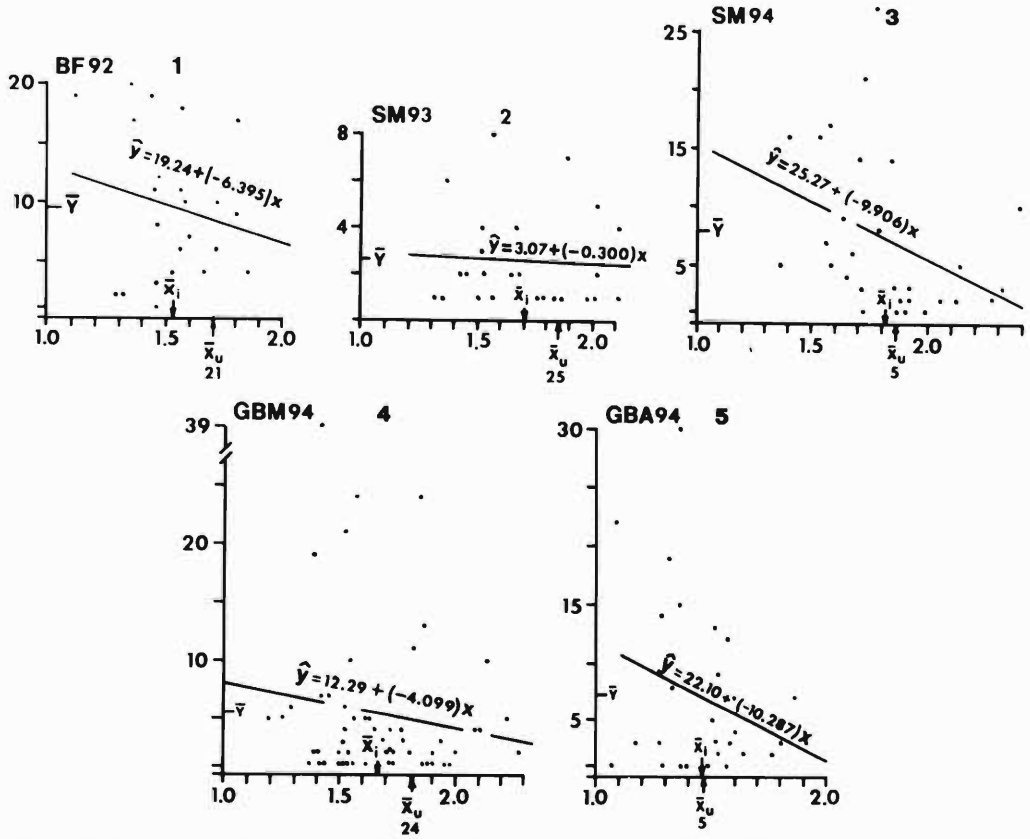
To evaluate the possible effect of *G. pennsylvaniensis* infections on spring peepers, we compared the weight of infected versus uninfected hosts. The test statistic for weight comparisons in each of 5 host populations was a t-test, with each test yielding a probability value. By combining probabilities and summing the natural log values for these 5 independent tests of significance, we were able to test the null hypothesis that mean infected host weight equaled mean uninfected host weight. It has been shown that $-2 \Sigma \ln P$ is distributed as χ^2 with 2k degrees of

Table 2. Mean intensities of *Flypthelmins pennsylvaniensis* in sample populations of *Pseudacris c. crucifer*. Means are significantly different ($P < 0.05$) from each other if 95% confidence limits (LL = lower limit; UL = upper limit) do not overlap.

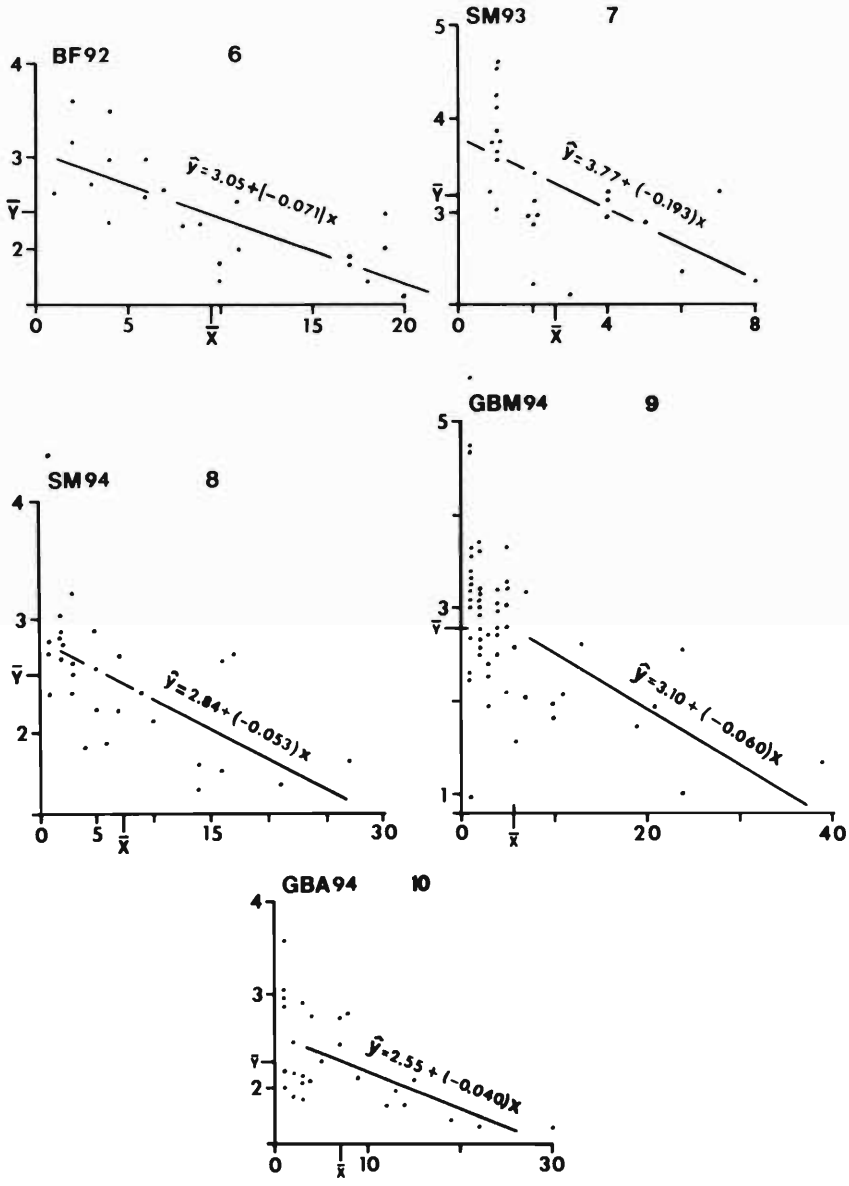
Host sample pop.	No. trematodes recovered	No. infected hosts	Intensity $\bar{x}$ (±1 SD)	95% confidence limits LL	UL
BF92	208	22	9.46 (6.29)	6.67	12.25
SM93	64	25	2.56 (2.06)	1.71	3.41
SM94	210	29	7.24 (6.88)	4.62	9.86
GBM94	300	55	5.46 (7.25)	3.50	7.42
GBA94	192	27	7.11 (7.50)	4.15	10.07

freedom, where k = the number of separate tests and probabilities (Sokal and Rohlf, 1981). Because our value of 22.344 for $-2 \Sigma \ln P$ was greater than $\chi^2_{.05[10]} = 18.307$, we rejected the null hypothesis and concluded that infected hosts were significantly smaller than their uninfected counterparts (Table 1).

There was an inverse relationship between host weight and numbers of *G. pennsylvaniensis* individuals in all 5 West Virginia host sample populations (Figs. 1–5). The slope of the regression line, however, was not significantly different from zero in 4 of those populations, allowing us to accept the null hypothesis (i.e., H_o: b = 0 for



Figures 1–5. Scatter diagrams showing numbers of *G. pennsylvaniensis* individuals (y-axis) as a function of host weight (g) (x-axis) for 5 West Virginia host collections. Each dot represents a single infected host. $\bar{Y}$ = mean number of *G. pennsylvaniensis* individuals. $\bar{x}_i$ = mean weight of infected hosts; $\bar{x}_u$ = mean weight of uninfected hosts. Numbers below the $\bar{x}_u$ notation represent the number of uninfected hosts. Differences between $\bar{x}_i$ and $\bar{x}_u$ are significant ($P < 0.05$) for hosts at BF92, SM93, and GBM94.



Figures 6–10. Scatter diagrams showing mean length (mm) of *G. pennsylvaniensis* individuals (y-axis) as a function of total numbers of *G. pennsylvaniensis* individuals (x-axis) in infected hosts for 5 West Virginia host collections. Each dot represents the number of digeneans (and their mean length) from a single infected host. $\bar{Y}$ = mean of mean length values; $\bar{x}$ = mean number of digeneans in infected hosts (i.e., $\bar{x}$ values here correspond to the $\bar{Y}$ values in Figs. 1–5).

trematode numbers as a function of host weight) in 4 of the 5 sample populations (Table 3). Similarly, Muzzall and Peebles (1991) found no relationship between helminth infections and length of *Rana sylvatica* and *P. c. crucifer* hosts.

There was also an inverse relationship between numbers of *G. pennsylvaniensis* individuals present in a given infected host and the mean length of those trematodes in all 5 West Virginia spring peeper sample populations (Figs. 6–10). The slope of the regression line was significantly different from zero for all of those populations, allowing us to reject the null hypothesis (i.e., H_o: $b = 0$ for mean trematode length as a function of total

Table 3. Significance of regression coefficients (i.e., b-values) for host weight (independent variable) versus the number of *G. pennsylvaniensis* individuals (dependent variable) (see Figs. 1–5).

Sample pop.	b-value	F [1, N − 2]	P
BF92	−6.395*	0.72 [1, 20]	>0.05
SM93	−0.300*	0.03 [1, 23]	>0.05
SM94	−9.906†	4.28 [1, 27]	<0.05
GBM94	−4.099*	1.08 [1, 53]	>0.05
GBA94	−10.287*	2.02 [1, 25]	>0.05

* Accept H_o: b = 0.
† Cannot accept H_o: b = 0.

trematodes) in all cases (Table 4). Such inverse relationships are not always evident. Nollen (1971) reported no relationship between numbers of *Philophthalmus megalurus* and their mean lengths. Later, however, Nollen (1983) found that *Philophthalmus gralli* in chickens were affected by crowded conditions, noting that adults "... from groups of over 40 per eye were significantly shorter than those in groups of up to 10 per eye." Fried and Nelson (1978) observed a similar effect with 2-wk-old *Zygocotyle lunata* individuals in chickens, where worms from single worm infections were more than twice as long as worms from initial infections of 100–500 cysts.

Information obtained during the course of this investigation corroborated some previously known aspects of *G. pennsylvaniensis* infections in spring peepers and revealed some new insights into this parasite/host relationship as well. Similar studies from other locations would be helpful in adding to our understanding of relationships between this digenean species and its amphibian host.

Table 4. Significance of regression coefficients (i.e., b-values) for number of *G. pennsylvaniensis* individuals (independent variable) versus their mean length (dependent variable) (see Figs. 6–10).

Sample pop.	b-value	F[1, N − 2]	P
BF92	−0.071*	26.71 [1, 20]	<0.001
SM93	−0.193*	12.15 [1, 23]	<0.005
SM94	−0.053*	15.37 [1, 27]	<0.001
GBM94	−0.060*	19.17 [1, 53]	<0.001
GBA94	−0.040*	13.83 [1, 25]	<0.005

* Cannot accept H_o: b = 0.

Acknowledgments

Collections of spring peepers were made under West Virginia Division of Natural Resources permit no. 21-1994. We thank Paul Nollen, Western Illinois University, for his helpful comments. Jeff May, Marshall University, advised on statistical procedures.

Literature Cited

Abdel-Malek, E. T. 1951. Menthol relaxation of helminths before fixation. Journal of Parasitology 37: 321.

Cheng, T. C. 1961. Description, life history, and developmental pattern of *Glypthelmins pennsylvaniensis* n. sp. (Trematoda: Brachycoeliidae), new parasite of frogs. Journal of Parasitology 47:469–477.

Coggins, J. R., and R. A. Sajdak. 1982. A survey of helminth parasites in the salamanders and certain anurans from Wisconsin. Proceedings of the Helminthological Society of Washington 49:73–83.

Fried, B. 1962. Growth of *Philophthalmus* sp. (Trematoda) in the eyes of chicks. Journal of Parasitology 48:395–399.

———, **and P. D. Nelson.** 1978. Host–parasite relationships of *Zygocotyle lunata* (Trematoda) in the domestic chick. Parasitology 77:49–55.

Joy, J. E., and B. T. Dowell. 1994. *Glypthelmins pennsylvaniensis* (Trematoda: Digenea) in the spring peeper, *Pseudacris c. crucifer* (Anura: Hylidae), from southwestern West Virginia. Journal of the Helminthological Society of Washington 61: 227–229.

Muzzall, P. M., and C. R. Peebles. 1991. Helminths of the wood frog, *Rana sylvatica,* and spring peeper, *Pseudacris c. crucifer,* from southern Michigan. Proceedings of the Helminthological Society of Washington 58:263–265.

Nollen, P. M. 1971. Studies on growth and infection of *Philophthalmus megalurus* (Cort, 1914) (Trematoda) in chicks. Journal of Parasitology 57:261–266.

———. 1983. The effects of crowding on adults of *Philophthalmus gralli* (Trematoda) grown in chickens. Journal of Parasitology 68:196–199.

Rankin, J. S. 1937. An ecological study of parasites of some North Carolina salamanders. Ecological Monographs 7:169–269.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry: The Principles and Practice of Statistics in Biological Research, 2nd ed. W. H. Freeman and Company, New York. 859 pp.

Systats Inc. 1992. Systat for Windows: Statistics. Version 5 ed. Systat, Inc., Evanston, Illinois. 750 pp.

Willey, C. H. 1941. The life history and bionomics of the trematode *Zygocotyle lunata* (Paramphistomidae). Zoologica 26:65–88.